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(21) International Application Number: PCT/US95/00055 (22) International Filing Date: 6 January 1995 (06.01.95) (30) Priority Data: 08/178,578 7 January 1994 (07.01.94) US (60) Parent Application or Grant (63) Related by Continuation US 08/178,578 (CIP) Filed on 7 January 1994 (07.01.94) (71) Applicant (for all designated States except US): SALIX PHARMACEUTICALS, INC. [US/US]; 3600 W. Bayshore Road, Palo Alto, CA 94303 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): JOHNSON, Lorin, K. [US/US]; 4979 Dolores Drive, Pleasanton, CA 94566 (US). SLEISENGER, Marvin, H. [US/US]; 415 Crown Road, Kentfield, CA 94904 (US). (74) Agents: PARK, Freddie, K. et al.; Morrison & Foerster, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: USE OF 2-HYDROXY-5-PHENYLAZOBENZOIC ACID DERIVATIVES AS COLON CANCER CHEMOPREVENTATIVE AND CHEMOTHERAPEUTIC AGENTS (57) Abstract <p>The present invention provides a method of colon cancer chemoprevention or chemotherapy comprising administering to an individual suffering from colon cancer or at risk to develop colon cancer a pharmaceutical composition comprising an effective amount of a 2-hydroxy-5-phenylazobenzoic acid derivative or an ester of an active metabolite or an oxidation product of an active metabolite thereof, or a pharmacologically acceptable salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof.</p> <div style="display: flex; justify-content: space-around;"> <div data-bbox="836 1155 1404 1501"> <p style="text-align: right;">CONTROL</p> </div> <div data-bbox="836 1627 1404 1963"> <p style="text-align: right;">BALSALAZIDE 250 mg/kg</p> </div> </div>		

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USE OF 2-HYDROXY-5-PHENYLAZOBENZOIC ACID DERIVATIVES AS
COLON CANCER CHEMOPREVENTATIVE AND CHEMOTHERAPEUTIC AGENTS

DESCRIPTION

Technical Field

5 This invention is related to colon cancer
chemoprevention and chemotherapy.

Background Art

Colon cancer currently accounts for 11% of all
deaths due to malignancy annually in the United States.
10 With an incidence of 62 per 100,000 and a prevalence of 300
per 100,000, the disease is currently the third leading
cause of death in men and the fourth leading cause of death
in women. Colon cancer has a particularly poor five-year
survival rate of less than 50%, due to the late stage at
15 which diagnosis is generally made. The currently favored
treatment, surgery combined with chemotherapy, has failed to
increase this survival rate. What is needed is a safe and
effective preventive therapy which could be initiated early
in patient populations known to be at an increased risk of
20 developing colon cancer.

Eicosanoids and Differentiated Functions of
Gastrointestinal Cells. Eicosanoids are significant
regulators of gastrointestinal epithelial cell growth,
differentiation and function. Eicosanoid products of the
25 prostaglandin series are known to induce mucus secretion
(Beckel and Kauffman (1981) Gastroenterology 80:770-776) and
the secretion of electrolytes and fluid (Miller (1983) Am.
J. Physiol. 245:G601-G623). They also induce active
transport (Bukhave and Rask-Madsen (1980) Gastroenterology

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78:32-37) and increase the replicative capacity of the epithelium (Konturek et al. (1981) Gastroenterology 80:1196-1201). These responses result in the maintenance of a differentiated, protective barrier system of tightly joined epithelial cells whose apical surface are covered by a dense glyco-conjugate chemical buffer. In the stomach and upper duodenum such a barrier protects against the acidic and proteolytic environment elaborated for digestion, while in the colon it protects against the invasion of bacteria and toxins. It is therefore not surprising that exogenous, synthetic prostaglandins are actively cytoprotective (Whittle and Vane (1987) In: Johnson (ed.) PHYSIOLOGY OF THE GASTROINTESTINAL TRACT, Vol. 1, 2nd ed., New York: Raven Press, pp. 143-180) and have found therapeutic utility as secondary anti-ulcer treatments. The gastrointestinal ("GI") system has therefore evolved to actively produce and rely on a specific differentiated complement of eicosanoid products present in the local environment. As all eicosanoids are derived from the common precursor arachidonic acid, which is itself liberated from membrane phospholipids, GI mucosal cells have a relatively high basal level of arachidonate turnover initiated by the enzyme phospholipase A₂ (PLA₂).

The GI system is also a primary defense mechanism against environmental bacteria, antigens and toxins and must therefore also possess the ability to mount an aggressive and rapid inflammatory response. This response also relies upon eicosanoid products of both the prostaglandin (PG) series as well as the chemotactic leukotriene series (LTs) and results in the influx of blood-borne neutrophils, macrophages and immune cells in response to the activating agent. Each of these invading cells also brings with it a capacity to metabolize its own phospholipids as well as mucosal and luminal phospholipids by releasing inflammatory (secreted)

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PLA₂ to amplify the release of arachidonic acid, which is then metabolized into both PGs and LTs.

Although the inflammatory cell infiltration confines and destroys the offending stimulus, the extent of damage due to inflammatory cell release products is significant. Neutrophils and macrophages release superoxide (O₂⁻) (Kitahora et al. (1988) Dig. Dis. Sci. 33:951-955) as well as hydrogen peroxide (H₂O₂) (Tauber and Babior (1985) Free Radic. Biol. Med. 1:265-307), and proteases (Ohlsson et al. (1977) Hoppe Seylers Z. Physiol. Chem 358:361-366). Where the resultant infiltration is extensive, significant denudation of the epithelial layer occurs with a subsequent compromise of the barrier function. Resolution of the inflammatory response is then required to regain final optimal epithelial barrier coverage.

Chronic GI Inflammation and GI Cancer Induction.

It is now well documented that chronic gastrointestinal inflammatory diseases such as ulcerative colitis (Lennard-Jones et al. (1977) Gastroenterology 73:1280-1285), Crohn's disease (Farmer et al. (1971) Cancer 28:289-295), and chronic atrophic gastritis (Sipponen et al. (1983) Cancer 52:1062-1067) are associated with an increased risk of subsequent gastrointestinal cancer. Although the mechanisms are not yet proven, three important intersecting pathways which could lead to subsequent transformation, heightened proliferation and malignant invasion occur during multiple acute inflammatory episodes. As discussed below these are: (1) increased colonic free radical and carcinogen load, (2) altered regulation of trophic eicosanoids, and (3) induction of gene products which mediate cellular invasion.

Altered carcinogen load due to inflammatory episodes. The colon may be uniquely exposed to a fairly high basal load of genotoxic carcinogens and tumor promoters resulting from the metabolism of dietary compounds and

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endogenous secretions such as bile acids by colonic bacteria. A relationship between fecal carcinogens and colon cancer induction is supported by findings of increased mutagens in the stools of high-risk individuals versus those of low-risk populations (Reddy et al. (1980) Mut. Res. 72:511-515). This correlation is also consistent with repeated findings showing a negative association between dietary fiber intake and incidence of colon cancer (Armstrong and Doll (1975) Int. J. Cancer 15:617-623). The protective effect of fiber is postulated to occur through an increase in stool volume which results in a dilution of stool carcinogens and decreased transmit time, leading to more rapid carcinogen elimination. These results raise the possibility that if a reduction in concentration of the stool carcinogen load can lead to a decreased cancer risk, then an increase in the carcinogen load may lead to an increased risk. One such increase in colonic carcinogens could be derived from successive inflammatory events.

A particularly relevant example is the inflammatory neutrophil production of carcinogenic nitrosamines via the L-arginine-dependent formation of nitrogen oxides as nitric oxide (Grisham (1993) Gastroenterology 104:A243). Other oxidative products released by inflammatory cells include superoxide as well as hydrogen peroxide, which in the presence of certain transition metals such as iron (Fe) can generate the highly reactive and cytotoxic hydroxyl radical (OH:) (Grisham (1990) Biochem Pharmacol 39:2060-2063). In addition to the increased carcinogen and free radical load elaborated by the influx of inflammatory cells, the arachidonic acid cascade is also known to be capable of producing mutagenic metabolites. A metabolite of prostaglandin H₂ (PGH₂), malondialdehyde (MDA), is a direct acting mutagen in vitro (Mukai and Goldstein (1976) Science 191:868-869) and a carcinogen in

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animals (Basu and Marnett (1983) Carcinogenesis 4:331-333) and can be enzymatically produced by thromboxane synthetase in high yields in cells with an active cyclooxygenase pathway. MDA has been shown to produce frameshift mutations similar to those associated with the human colon p53 gene (Marnett, et al. (1985) Mutat. Res. 129:36-46). PGH synthase itself is a potent peroxidase and has been shown to catalyze the activation of a wide range of polycyclic hydrocarbons to mutagens (Marnett (1981) Life Sci. 29:531-546).

These findings suggest that chronic and aberrant (inflammatory cell driven) activation of the arachidonic acid cascade in the gastrointestinal tract is one pathway which can lead to an increased carcinogen load with the potential induction of DNA mutations during times of maximum DNA synthesis. Increased cell proliferation, which follows epithelial denudation induced by invading inflammatory cells, could lead to an increased number of cells susceptible to the action of such carcinogens. Alternatively, increased proliferation could serve to amplify mutations (through clonal expansion) induced previously by carcinogens.

Altered eicosanoid regulation can drive proliferation due to inflammatory episodes. An additional mechanism linking GI inflammation and the progression of GI cancers could be a disruption in normal eicosanoid regulation. As discussed above, the normal differentiated functions of the GI mucosal epithelium are intimately linked to the diverse range of biological activities affected by endogenous eicosanoids. Since these agents act locally and generally have short half-lives due to active metabolic inactivation, periods of acute inflammatory events will dramatically alter the normal regulation of eicosanoid homeostasis.

Upon neutrophil and macrophage invasion into an inflammatory site these normal dynamics are dramatically

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altered. First, inflammatory cells bring with them a wide range of additional agonists such as cytokines, proteases and growth factors (Adams and Hamilton (1984) Ann. Rev. Immunol. 2:283-318, Ohlsson et al. (1977) Physiol. Chem. 358:361-366, Nathan and Cohn (1980) In: Kelly et al. (eds.), Textbook of Rheumatology, New York: W. B. Saunders, pp. 186-215), which by themselves chronically activate cellular PLA₂ (cPLA₂) to release arachidonic acid. Second, inflammatory cells are a rich source of additional forms of PLA₂ known as secretory or sPLA₂ (Seilhamer et al. (1989) J. Biol. Chem. 264:5335-5338). whose activities in GI inflammatory disease has recently been documented (Minami et al. (1992) Gut 33:914-921).

Unlike cPLA₂, sPLA₂ is released from inflammatory cells (Wright et al. (1990) J. Biol. Chem. 265:6675-6681), platelets (Hayakawa et al. (1988) J. Biochem. 104:767-772), chondrocytes (Lyons-Goirdano et al. (1989) Biochem. Biophys. Res. Commun. 164:488-495) and smooth muscle cells (Nakano et al. (1990) FEBS Lett. 261:171-174) by cytokines (Pfeilschifter et al. (1989) Biophys. Res. Commun. 159:385-394), and, in particular, by endotoxin (Oka and Arita (1991) J. Biol. Chem. 266:9956-9960). In addition, because the extracellular milieu contains maximal calcium concentrations, sPLA₂ is unregulated once released. Upon release it therefore actively hydrolyses arachidonic acid and other fatty acids from the sn-2 position of phospholipids found in cellular and bacterial membranes as well as those from dietary and lipoprotein sources. The lysophospholipid resulting from removal of the sn-2 fatty acid from many such phospholipids is potently lysogenic for surrounding cells (Okada and Cyong (1975) Jpn. J. Exp. Med. 45:533-534). Thus, this reaction can also lead to epithelial cell lysis and denudation in the unfiltered area, ultimately requiring enhanced proliferation to maintain the barrier function.

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Inflammatory response and activation of genes directing cellular invasion. The inflammatory response not only disrupts normal eicosanoid regulation but also leads to the activation of gene products required for cellular invasion. One such product, the urokinase plasminogen activator receptor (uPAR), is normally expressed by intestinal epithelial cells. Its anchorage to the cell surface may be an important determinant of normal crypt cell migration and desquamation via cell surface proteolysis (Kristensen et al. (1991) J. Cell. Biol. 115:1763-1771). In inflammatory cells, uPAR gene expression is induced by activators of protein kinase C, by tumor promoters such as the phorbol ester TPA (Lund et al. (1991) J. Biol. Chem. 266:5177-5181), and by various cytokines (Lund et al. (1991) EMBO J. 10:3399-3401) which induce an invasive phenotype required for tissue infiltration (Stoppelli et al. (1985) Natl. Acad. Sci. USA 82:4939-4943). It is therefore not surprising that high levels of uPAR expression have also been detected in several tumor cell lines with metastatic potential, including colon cancer-derived cells (Pyke et al. (1991) Am. J. Pathol. 138:1059-1067). Of particular interest are co-culture experiments showing that invasive potential was more highly correlated with expression of uPAR than its ligand plasminogen activator (Ossowski et al. (1991) J. Cell. Biol. 115:1107-1112). Thus, multiple cycles of inflammatory responses could also contribute to uPAR overexpression in colonic mucosal cells, resulting in the acquisition of an invasive phenotype in a previously benign tumor.

GI mucosal cells may therefore be uniquely sensitive to chronic inflammatory episodes because of three intersecting pathways: (1) the epithelial cells are positioned in an environment with a high carcinogen load which can further increase during inflammatory episodes; (2) the products of both the endogenous and infiltrated

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eicosanoid cascades are trophic agents; and (3) their own differentiated response to inflammatory agents includes expression of gene products required for acquisition of an invasive phenotype. Together these three paths could lead to a transformation event and resultant tumor induction, progression and invasion. Therefore, agents which block certain arms of the eicosanoid cascade are useful in colon cancer chemoprevention.

Polyps and Aberrant Crypt Foci as Precursors of Colon Cancer. It is now widely held that adenomatous polyps are precursors of colorectal cancer and their appearance, size and multiplicity are predictive of the relative risk of developing colon cancer (see Lotfi et al. (1986) Mayo Clinic Proc. 61:337-343). While adenomatous polyps are precursor lesions of colon cancer, it is also now accepted that certain early pathological lesions, termed aberrant crypt foci are precursor lesions to adenomatous polyps. Aberrant crypt foci (ACF) are identifiable in normal-appearing human colon mucosa and have been shown to be present in greater number and size in specimens from patients with sporadic colon cancer or generically inherited familial adenomatous polyposis (Ronucci et al. (1991) Human Pathol. 22(3):287-294; Pretlow et al. (1991) Cancer Res. 51:1564-1567).

NSAIDs and Colon Cancer Chemoprevention. There is evidence that several non-steroid antiinflammatory drugs (NSAIDs) are effective in reducing the number of tumor-bearing animals and tumor incidence per animal in rat models of colon carcinogenesis. (Narisawa et al. (1981) Cancer Res. 41:1954-1957), (Pollard et al. (1983) Cancer Lett. 21:57-61) (Moorghen et al. (1988) J. Pathol. 156:341-347) (Reddy et al. (1993) Gastroenterology 104:A443) where up to a 70% reduction in tumor incidence has been noted at doses of 80% of the maximum tolerated dose. In a study of dimethylhydrazine-induced colon carcinogenesis, sulindac was observed to reduce

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tumor incidence only when present during carcinogen administration, but not if given 17 weeks following the carcinogen (Moorghen et al. (1988) J. Pathol. 156:341-347).

5 In several retrospective studies, aspirin intake has been assessed as a chemopreventive therapy in ensuing colon cancer incidence. The results of these studies have ranged from a halving (Kune et al. (1988) Cancer Res. 48:4399-4404) of the risk of colon cancer development to a 50% increased risk (Paganini-Hill et al. (1991) J. Natl. Cancer Inst. 83:1182-1183). It should be noted, however, that any human study employing aspirin and other NSAIDs, especially retrospective studies, could be flawed by the induced frequency of GI bleeding which may allow for earlier detection of the polyp or tumor in the NSAID group through
10 occult blood screening and sigmoidoscopy.
15

Although the retrospective aspirin studies have provided equivocal results, the initial results for certain NSAIDs found in animal studies, summarized above, have been replicated in prospective human trials. The NSAID, sulindac, has been shown in a randomized, placebo-controlled, double-blind crossover study to cause regression of polyps in nine familial polyposis patients in less than 4 months (Labayle et al. (1991) Gastroenterology 101:635-639). In addition, polyp growth resumed following sulindac withdrawal. This
20 finding is significant because a similar study using indomethacin found no influence on polyp regression (Klein et al. (1987) Cancer 60:2863-2868). Although both of these NSAIDs derive their anti-inflammatory activity from inhibition of cyclooxygenase, sulindac is a prodrug which is
25 converted to its active metabolite, sulindac sulfide, by the colonic bacteria (Shen and Winter (1975) Adv. Drug. Res. 12:89-245). In contrast, indomethacin is ingested in its active form, where it is absorbed primarily from the upper GI tract for systemic delivery (Hucker et al. (1966)
30

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J. Pharmacol. Exp. Ther. 153:237-249). It is therefore likely that the concentrations of active metabolite delivered by sulindac to the colon are significantly higher than from indomethacin. This result suggests that a significant
5 portion of the observed NSAID chemopreventive effect is derived from local action of the drug at the mucosal-lumen interface.

Although this evidence of NSAID-induced inhibition of colonic tumor incidence in animal models suggests a
10 mechanism of inhibition via the tumor cell cyclooxygenase, confirmation of such a mechanism is lacking. Tumor tissue excised from NSAID-treated animals has been shown to secrete dramatically reduced levels of PGE₂, which is consistent with this hypothesis (Reddy et al. (1992) Carcinogenesis 13:1019-
15 1023). However, most colonic tumors are heterogeneous with respect to their resident cell types, and several reports have documented that epithelial cell lines generated from colorectal adenocarcinomas are not high producers of PGE₂ or other prostanoids (Hubbard et al. (1988) Cancer Res. 48:4770-
20 4775). A report on the eicosanoid production of cells fractionated from human colon tissue, however, showed that tumor epithelial cells produced PGE₂ levels similar to uninvolved tissue, while tumor-derived mononuclear cells showed significantly greater eicosanoid synthesis than their
25 normal counterparts (Maxwell et al. (1990) Digestion 47:160-166). Therefore, target cells sensitive to NSAID inhibition may not be the tumor epithelial cells, but could be some other resident producing high PG levels to which the epithelial cells are responsive.

30 Profile of the Preferred Chemopreventive Therapy
All NSAIDs carry significant side effect profiles. NSAIDs are not tissue specific in their inhibition of cyclooxygenase products, and both renal and gastrointestinal systems are particularly sensitive. NSAIDs reduce renal perfusion

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resulting in nephrotoxicity (Clive and Stoff (1984) N. Engl. J. Med. 310:563-572), and because prostaglandins are necessary for the normal differentiated functions of the GI epithelium, NSAID induced gastric ulceration is a significant contributor to morbidity and mortality associated with this class of drugs (Langman (1989) Gastroenterology 96:640-646, Bjarnason et al. (1992) Gastroenterology 104:1832-1847). In the lower bowel, chronic NSAID therapy has been reported to result in colitis ranging from proctitis to pancolitis (Tanner and Raghunath (1988) Digestion 41:116-120). In one retrospective study, it was estimated that NSAID ingestion was found in 25% of patients presenting with large and small bowel perforation and bleeding (Langman et al. (1985) Br. Med. J. 290:347-349). Finally, in patients with existing chronic inflammatory bowel diseases, who are already at high risk of developing colorectal cancer, non-5-ASA-containing NSAIDs as well as aspirin therapy is contraindicated due to exacerbation of the existing disease condition (Rampton and Sladen (1981) Prostaglandins 21:417-425). The ideal drug for colon cancer chemoprevention is, therefore, one with the following profile:

1) Colon specific - It should be a prodrug with no activity elaborated in the upper GI tract, but converted to active form upon reaching the colon (sulindac-like);

2) Limited absorption - Absorption of the parent and metabolites should be minimal, especially once converted to its active form;

3) Lack of systemic activity - Once absorbed, metabolic inactivation should convert the drug to an inactive form, thereby limiting systemic effects on the renal and GI systems;

4) Antioxidant properties - Colon-specific antioxidant activity would further serve to lower the carcinogen burden; and

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5) NSAID-like anti-inflammatory mechanism - The active metabolite should inhibit inflammation-induced eicosanoid pathways, however, eicosanoid inhibition without compromise to basal maintenance pathways would be preferred.

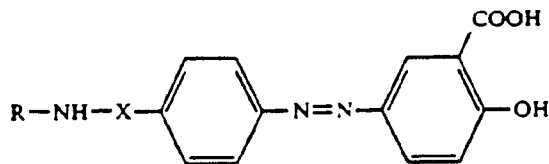
5 To summarize, certain NSAIDs have been shown to inhibit colon tumor incidence in carcinogen-induced animal models and inhibit polyp growth in humans. Although the mechanism for tumor inhibition by NSAIDs is unproven, these drugs may modulate gastrointestinal eicosanoid production and metabolism. Unfortunately, NSAIDs also have a disturbing and serious gastrointestinal side effect profile which may preclude their chronic use. Therefore, it would be highly desirable to identify NSAIDs which are effective as chemopreventive agents but which lack these side effects.

10 Chan, U.S. Patent No. 4,412,992, describes the preparation of 2-hydroxy-5-phenylazobenzoic acid derivatives and their use in the treatment of ulcerative colitis.

DISCLOSURE OF THE INVENTION

20 In accordance with one embodiment of the present invention, there is provided a method of treating an individual suffering from colon cancer or at risk to develop colon cancer comprising administering to the human an effective amount of a pharmaceutical composition comprising a 2-hydroxy-5-phenylazobenzoic acid derivative of the general

25 formula:



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wherein X is an $-SO_2-$ or $-CO-$ group and R is either a phenyl or carboxymethylphenyl radical or is a radical of the formula $-(CH_2)_n-Y$, in which Y is a hydroxyl group, an amino group, a monoalkyl- or dialkyl-amino group, the alkyl moieties of which contain up to 6 carbon atoms, or a carboxylic or sulphonic acid group, and n is a whole number of from 1 to 6, and in which one or more of the hydrogen atoms in the alkylene radical can be replaced by amino groups, monoalkyl- or dialkyl-amino groups, the alkyl moieties of which contain up to 6 carbon atoms, or alkyl radicals, and in which the $-(CH_2)_n-Y$ radical is either attached directly to the nitrogen atom or via a benzene ring, with the proviso that $R-NH-X-$ is other than a $-CO-NH-CH_2-COOH$ radical; or an ester or an active metabolite or an oxidation product of an active metabolite thereof, or a non-toxic, pharmacologically acceptable salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof.

In accordance with another embodiment of the present invention, the pharmaceutical composition of the method consists essentially of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof or a salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite thereof in admixture with a solid or liquid pharmaceutical diluent or carrier.

In yet another embodiment of the present invention, the 2-hydroxy-5-phenylazobenzoic acid derivative is balsalazide.

In a further embodiment of the present invention, the active metabolite is 5-ASA.

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In yet a further embodiment of the present invention, the oxidation product of an active metabolite is an oxidation product of 5-ASA.

5 In a further embodiment of the present invention, the oxidation product of 5-ASA is gentisic acid or 5-nitro-salicylate.

10 In still a further embodiment of the present invention, the pharmaceutical composition is administered orally to an individual suffering from or at risk to develop colon cancer in a daily dosage ranging from 1 to 14g per 70 kg body weight per day of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof or a salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof.

15 In another embodiment of the present invention, the pharmaceutical composition is administered rectally to an individual suffering from or at risk to develop colon cancer in a daily dosage ranging from 1 to 14g per 70 kg body weight per day of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof or a salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

30 Figure 1 shows the chemical structure of a 2-hydroxy-5-phenylazobenzoic acid derivative wherein the generic name is balsalazide. The aminosalicylate moiety, 5-aminosalicylic acid (5-ASA) is linked to a carrier molecule, 4-aminobenzoyl- β -alanine (4-ABA), through an azo-bond.

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Figure 2 shows the effects of 5-ASA on proliferation of the adenocarcinoma cell line HT-29 at doses of 0, 0.1, 1.0 and 10 mM. The cell numbers shown are the average of triplicate experiments.

5 Figure 3 shows the effects of 5-ASA on proliferation of the adenocarcinoma cell line LS174T at doses of 0, 0.1, 1.0 and 10 mM. The cell numbers shown are the average of triplicate experiments.

10 Figure 4 shows an analysis of the number of aberrant colonic crypts induced by treating groups of rats with the carcinogen, azoxymethane in the presence and absence of balsalazide administered in the drinking water.

Figure 5 shows the relative inhibitory responses produced by balsalazide and 5-ASA at lower concentrations.

15 Figures 6A and 6B show the distribution of aberrant crypt foci throughout the colon of control animals and AOM (20mg/kg) injected animals, 6 weeks following the second injection, respectively. The number of foci containing 1, 2, 3, 4 or 5 or more are shown (lower to upper
20 curves in each panel, respectively).

Figure 7 shows the inhibition of LS174T cell proliferation by various concentrations of 5-ASA dissolved in media 4 days prior to exposure to the cells (black) or 5-ASA at 10 mM dissolved in media immediately prior to exposure to
25 the cells (white). Cells were grown an additional 4 days prior to quantitation.

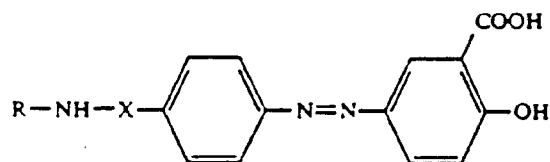
Figures 8A and 8B show the inhibition of colon cancer cell proliferation by various concentrations of two oxidation products of 5-ASA, gentisate and 5-nitro-salicylate, respectively.
30

MODES FOR CARRYING OUT THE INVENTION

2-hydroxy-5-phenylazobenzoic acid derivatives, particularly balsalazide and its active metabolites, have

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been found to be effective in colon cancer chemoprevention. These 2-hydroxy-5-phenylazobenzoic acid derivatives have the following general formula:



wherein X is an $-SO_2-$ or $-CO-$ group; and R is either a phenyl
 5 or carboxymethylphenyl radical or is a radical of the formula
 $-(CH_2)_n-Y$, in which Y is a hydroxyl group, an amino group, a
 monoalkyl- or dialkyl-amino group, the alkyl moieties of
 which contain up to 6 carbon atoms or a carboxylic or
 sulphonic acid group, and n is a whole number of from 1 to 6,
 10 and in which one or more of the hydrogen atoms in the
 alkylene radical can be replaced by amino groups, monoalkyl-
 or dialkyl-amino groups, the alkyl moieties of which contain
 up to 6 carbon atoms or alkyl radicals, and in which the -
 $(CH_2)_n-Y$ radical is either attached directly to the nitrogen
 15 atom or via a benzene ring, with the proviso that R-NH-X- is
 other than a $-CO-NH-CH_2-COOH$ radical.

As employed herein, the term "2-hydroxy-5-phenylazobenzoic acid derivative" also embraces esters or
 active metabolites of the compound or non-toxic,
 20 pharmacologically acceptable salts of the compound or its
 esters or active metabolites.

The term "active metabolite" refers to the
 products of the metabolism of 2-hydroxy-5-phenylazobenzoic
 acid derivatives in the human body, e.g., via the action of
 25 colonic bacteria, which inhibit colon cancer cell
 proliferation. For example, as discussed below, 5-ASA is an
 active metabolite of balsalazide.

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The term "oxidation product" refers to products produced by exposing active metabolites of 2-hydroxy-5-phenylazobenzoic acid derivatives, for example, 5-ASA, to oxidizing conditions such as hypochlorite or hydrogen peroxide.

Balsalazide sodium is a colonic-specific, non-steroidal, anti-inflammatory aminosalicylate which is useful in the treatment of active ulcerative colitis. Balsalazide as well as one of its primary metabolites inhibits the growth of cultured human colon cancer cells and balsalazide inhibits aberrant crypt formation in animals treated with the carcinogen, azoxymethane. Like other NSAIDs, balsalazide is effective in human colorectal cancer chemoprevention. However, balsalazide has three important safety advantages: (1) the delivery of the drug is colon-specific; (2) no evidence of gastric or duodenal ulceration has been observed; and (3) no evidence of nephrotoxicity has been reported among over 500 patients treated to date, some for as long as three years. Balsalazide therefore possesses an ideal combination of efficacy and safety for the chemoprevention of colon cancer.

Colon-specific delivery. Balsalazide is a prodrug the inactive form of which, like sulindac, is converted to an active antiinflammatory drug by the action of colonic bacteria. As shown in Figure 1, balsalazide links the aminosalicylate 5-aminosalicylic acid (5-ASA) to an inert carrier molecule, 4-aminobenzoyl- β -alanine (4-ABA), through an azo-bond. Bacterial azoreductase hydrolyses this bond, releasing the 5-ASA for local action.

Limited systemic absorption. Balsalazide, when taken orally, passes uncleaved and virtually unabsorbed through the upper GI tract. As little as 0.3% of the ingested dose of the prodrug is found in plasma or urine, with 99% of the drug reaching the colon intact. In the

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colon, the drug undergoes hydrolysis to form 5-ASA and 4-ABA, which interact with the colonic mucosa and are converted to their N-acetyl forms. The majority of 5-ASA resulting from a single dose of balsalazide is converted over a 96-hour period to N-acetyl-5-ASA (Nac5ASA) and excreted in the urine, while the 4-ABA and its N-acetyl metabolite are poorly absorbed. Since the N-acetyl metabolite of 5-ASA is inactive, systemic activity of delivered 5-ASA is also reduced (Chan et al. (1983) Dig. Dis. Sci. 28: 609-615).

Antioxidant properties. As discussed previously, a chemopreventive agent which possesses effective antioxidant properties can also contribute to a reduction in the colonic carcinogen load as well as reducing the damage induced by free radical release from invading inflammatory cells. 5-ASA inhibits nitrosamine formation from arginine-dependent nitric oxide (Grisham (1993) Gastroenterology 104:A243). In addition, both intact balsalazide and 5-ASA are effective free radical scavengers against O_2^- and $OH\cdot$. Balsalazide and 5-ASA, when incubated with rectal biopsies from patients with ulcerative colitis, reduce rectal mucosal reactive oxygen metabolite production by over 90%. Both agents are also more effective than the anti-oxidants taurine, ascorbate or N-acetyl cysteine when used at similar concentrations (Simmonds et al. (1992) Gastroenterology 102: A696).

Colonic antiinflammatory activity without upper GI effects. Balsalazide possesses anti-inflammatory activity in several animal models of colonic inflammation, as well as in patients with active (Green et al. (1993) Gastroenterology 104:A709) and quiescent (Gaiffer et al. (1992) Ailment. Pharmacol. Ther. 6:479-485) ulcerative colitis. Importantly, the drug displays excellent gastrointestinal tolerance.

Balsalazide and 5-ASA inhibit colon cancer cell proliferation in vitro. 5-ASA, as well as balsalazide, show

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significant growth inhibitory activity against human colon cancer cells.

Oxidation products of 5-ASA inhibit colon cancer cell proliferation in vitro. Oxidation products of 5-ASA show surprisingly unexpected growth inhibitory activity against human colon cancer cells when compared to 5-ASA.

Methods of Colon Cancer Chemoprevention and/or Chemotherapy.

2-hydroxy-5-phenylazobenzoic acid derivatives of the present invention, particularly balsalazide and its metabolites, are, therefore, useful for colon cancer chemotherapy. They will preferably be administered to human individuals suffering from colon cancer or at risk to develop colon cancer in the form of pharmaceutical compositions comprising the 2-hydroxy-5-phenylazobenzoic acid derivative(s). The pharmaceutical composition may also comprise one or more non-toxic pharmaceutically acceptable carriers, excipients, and/or diluents. Oral administration is preferred. Standard pharmaceutical formulation techniques are used, such as those disclosed in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA, latest edition.

The compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations. Tablets and capsules for oral administration may be in a form suitable for unit dose presentation and may contain conventional excipients. Examples of these are: binding agents such as syrup, acacia, gelatin, sorbitol, tragacanth, and polyvinylpyrrolidone; fillers such as lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricants, such as magnesium stearate, silicon dioxide, talc, polyethylene glycol or silica; disintegrants, such as potato starch; or acceptable wetting agents, such as sodium lauryl sulfate. The tablets may be

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coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents*, e.g., sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats, emulsifying agents, e.g., lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (including edible oils), e.g., almond oil, fractionated coconut oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives such as methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavoring or coloring agents.

The percentage of active material in the pharmaceutical compositions of the present invention may be varied, it being necessary that it should constitute a proportion such that a suitable dose for the desired therapeutic effect shall be obtained. In general, the preparations of the present invention should be administered orally or rectally to humans to give 1 to 14 g of active substance per day per 70 kg of body weight.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1: Balsalazide and 5-ASA inhibit colon cancer cell proliferation in vitro

The activity of balsalazide and its metabolite 5-ASA on the growth of human colon cancer cells was demonstrated *in vitro* using the human adenoma cells HT-29 and LS174T. Cells were grown in Dulbeccos Modified Eagle medium

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supplemented with 10% calf serum in an atmosphere of 5% CO₂ at 37°C. For growth experiments, cells were seeded into 24-well tissue culture dishes (2cm²/well) at a density of 20,000 cells/well and allowed to attach for 12 hours. The growth medium was then changed and replaced with fresh media containing the test drugs.

The test drugs were dissolved in tissue culture media at the desired concentration and added to individual wells. To determine the number of cells per well, media was removed and the cells washed with phosphate buffered saline solution. The cells were then incubated 15 minutes with trypsin-EDTA solution until detached. The detached cells were then removed from the wells and counted using a Coulter Counter (Model ZBI). As shown in Figures 2 and 3, both human colon cancer cell lines HT-29 and LS174T were growth inhibited by progressively greater concentrations of 5-ASA. At a dose of 10 mM 5-ASA inhibition reached 82% and 85%, respectively.

Comparative studies were also carried out to test the effectiveness of balsalazide with its two metabolites, 5-ASA and 4-ABA as well as with acetyl salicylic acid (aspirin) on cancer cell proliferation. As shown in Table I, acetylsalicylic acid, which covalently inhibits cyclooxygenase, was potently inhibitory in this assay, as was the parent drug balsalazide. However, 4-ABA, the carrier molecule of balsalazide, was inactive. The cell numbers shown in Table I are the average of triplicate culture wells.

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TABLE I

<u>Condition</u>	<u>Dose</u>	<u>HT-29</u>		<u>LS174T</u>	
		<u>Cell No.</u> <u>on Day 10</u>	<u>% of Control</u> <u>Cells</u>	<u>Cell No.</u> <u>on Day 10</u>	<u>% of Control</u> <u>Cells</u>
Control	-	1315800	-	709750	-
Ac-sali- cyclic acid	(10 mM)	14625	1.11	8300	2.24
Balsal- azide	(10 mM)	47500	3.60	24925	3.51
5-ASA	(10 mM)	512825	38.97	8675	1.22
4-ABA	(10 mM)	1316625	100.06	768700	108.30

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Further studies designed to examine the dose response relationship of the growth inhibitory effect seen with balsalazide and 5-ASA showed that both compounds produced similar results when examined using either HT-29 cells or LS174T cells. For dose response studies a sensitive dye binding assay was adapted which allows simultaneous assay of multiple agents and doses in 96-well plates. Cells were plated and allowed to adhere for 24 hours prior to addition of the study drug. Cell densities were then determined 4 days later by fixation of the wells and staining with Sulforhodamine B dye. Optical density of the stained cultures was then determined using a 96-well plate reader at 495 nm (Skehan et al. (1990) J. Natl. Cancer Inst. 82:1107-1112).

As shown in Figure 5, balsalazide consistently produced a greater inhibitory response at lower concentrations than did 5-ASA. Using HT-29 cells the IC_{50} values were 5.7 mM and 11.8 mM respectively while using LS174T cells the respective IC_{50} values were 5.2 mM and 11.5 mM. At the lowest concentrations of 5-ASA tested (0.1-1.0 mM for LS174T and 0.1-4 mM for HT-29), a reproducible and statistically significant increase in cell number of approximately 20% was observed with both cell types. This is consistent with previously published results by others who observed an increase in protein synthesis induced by other NSAIDs at low concentrations followed by an inhibition at higher concentrations (Hial et al. (1977) J. Pharm. Exp. Therap. 202:446-452).

Example 2: Influence of balsalazide and metabolites on aberrant crypt formation in the rat

Aberrant crypts were originally described in carcinogen-induced colonic mucosa as crypts of "increased

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size, thicker epithelial lining and increased pericryptal zones" (Bird et al. (1989) Cancer Surv. 8:189-200). NSAIDs are among the most potent inhibitors of aberrant crypt formation induced by the carcinogen, azoxymethane (AOM) in the rat (Wargovich et al. (1992) J. Cell. Biochem. 16G:51-54). The anti-tumor activity of several of these agents has been confirmed in longer term AOM animal studies. The aberrant crypt model is, therefore, an ideal in vivo assay for efficacy of balsalazide and metabolites.

Inhibition of Aberrant Crypt Induction. The efficacy of balsalazide (BSZ), and its metabolites, 5-aminosalicylic acid (5-ASA) and 4-aminobenzoyl- β -alanine (4-ABA) on the inhibition of aberrant crypt formation was determined in a model of colon cancer induced by azoxymethane (AOM) in the rat.

Four groups of 5 male F344 rats were used. Animals were 6-8 weeks of age at the start of dosing with a starting weight range within +/-20% of the overall mean. The expected response to AOM treatment from published studies is the formation of 100-140 aberrant crypts per animal (Wargovich et al. (1992) J. Cell. Biochem. 16G:51-54). Using these response rates and a chi square analysis with 95% confidence limits, the sample size of 5 animals per group allows detection of statistically significant inhibition (at the $p < 0.05$ level) by the test drugs when up to 50-75 aberrant crypts per animal are observed.

Animals were acclimatized for 10 days prior to being randomized into the test groups by body weight classes. Test drug was dissolved in the drinking water at a concentration which gave the desired dose per animal per day based on an average water consumption of 50 ml per day per animal. Two groups were allowed one week of drug treatment prior to the AOM injections. Carcinogenesis was induced by two subcutaneous injections of AOM at 20 mg/kg given at the

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start of week 2 and week 3. Test article dosing then continued until the end of week 5.

5 The choice of dose level for balsalazide is made by review of other preclinical studies for efficacy in colitis models. A dose of 200 mg/kg/day in a 500 g rat equates to a dose of 14 g per day in a 70 kg human, approximately twice the therapeutic dose for treatment of active ulcerative colitis and over 4-fold the dose for maintaining remission. This dose level results in colonic
10 concentrations of 5-ASA of 5-20 mM. The maximum tolerated dose of balsalazide in the rat is in excess of 12,000 mg/kg/day.

Body weights were recorded twice weekly, and water consumed by each cage of animals measured twice weekly.
15 Consumption is calculated as average ml/animal/cage.

For aberrant crypt analysis, animals were sacrificed under CO₂ euthanasia at the end of either three weeks or five weeks of dosing. The large intestine was excised at the caecum and anus, removed and opened
20 longitudinally. The tissue was freed from contents, rinsed in saline and fixed at 4°C for two hour in 2% paraformaldehyde and stained with 0.2% methylene blue for 3-5 minutes. After a saline rinse, the number of foci of aberrant crypts was scored by counting under a microscope at
25 40-100X magnification. The length of the colonic tissue was also measured. A representative sample of the grossly identified lesions was embedded in glycol methacrylate at 4°C; 3-4 μ m sections of each foci are stained with hematoxylin, eosin and azure (HEA) for histological
30 evaluation.

For gastroduodenal erosion analysis, the fundus, antrum, and duodenum were removed and processed separately as above. Gastric erosions were scored using the gastric

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erosion index of length and severity as described (Peskar et al. (1986) Prostaglandins 31:283-287).

The results are shown in Figure 4 where the average number of aberrant crypts per longitudinal cm of colonic tissue is shown for animals examined either one or three weeks following two subcutaneous injections of azoxymethane. At both time points the animals treated with balsalazide had a greatly reduced average number of aberrant crypts than the control animals. At week 1 there was a 61.8% inhibition and at week five there was a 58.1% inhibition in the average number of aberrant crypts.

Inhibition of Aberrant Crypt Progression. The data shown in Figure 4 were collected from the distal 7-8 cm of colonic tissue. At these early time points, this area contained over 80% of the total aberrant crypt foci. An additional study was therefore designed to examine a longer time point, assess the distribution throughout the entire 25 cm of colon and determine if the drug treatment could be initiated after the second AOM injection. This later objective is of particular interest because it is important to demonstrate that the chemopreventive agent is inhibiting the progression of aberrant crypt growth following the initial transformation event by the carcinogen.

In the second study, groups of 8 animals received two injections of azoxymethane one week apart. Balsalazide treatment was then initiated with one group 24 hours following the second injection. Six weeks later the animals were processed for aberrant crypt analysis. Crypts were stained in situ by rectal instillation of a 0.2% solution of methylene blue for 15 min under anesthesia. Animals were then exsanguinated, colonic tissue from the anus to the caecum was excised, cleaned and fixed for 2 hours in 2% paraformaldehyde. Crypts were counted by viewing at a magnification of 40x. In addition to collecting data on the

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number of foci, data was also collected on the multiplicity of aberrant crypts per foci. These data are shown in Figure 6. As is evident, even when balsalazide was given beginning 24 hours following the second AOM injection a dramatic inhibition of aberrant crypt foci was observed which was comparable to the previous experiment (approximately 60% inhibition overall) illustrated in Figure 4.

Previous studies by Pretlow et al (Pretlow et al. (1992) Carcinogenesis 13:1509-1512) have reported on the relationship between aberrant crypt multiplicity and ultimate tumor development using the same carcinogenesis model. In those studies the number of aberrant crypt foci containing 4 or more aberrant crypts strongly correlated with tumor formation suggesting that these larger foci are the ones more likely to progress on to tumors. The data from the above experiment was analyzed to determine the effect of balsalazide on the aberrant crypt multiplicity. These data are shown below in Table II and reveal that balsalazide exerts a greater inhibition of the number of foci containing 4 or more aberrant crypts than those with less than four. For foci with 1-3 crypts the mean inhibition ranged from 57-61% ($p < 0.003$, Fischer exact test) while the number of foci with 4 or more crypts was reduced by a mean of 72-76% ($p < 0.001$). When the results on the number of foci containing 4 or more crypts are compared with those containing 3 or less the difference remains statistically significant at the $p < 0.022$ level using the Fischer exact test.

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TABLE II

Balsalazide Inhibition of Aberrant Crypt Multiplicity.
 The numbers shown are the mean number of foci of various
 sizes
 in each group of 8 animals measured 6 weeks after the second
 AOM injection.

Multiplicity	1	2	3	4	5 to 8	Total
BSZ	10.3	19.1	15.3	8.5	9.6	63.75
Control	24	48.8	37.3	30.5	40.6	181.75
% Inhibition	57±23%	61±16%	59±17%	72±19%	76±12%	65±10%
Fischer	0.001	0.001	0.003	0.0001	0.0001	0.001

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These results show that foci containing the greatest number of aberrant crypts are reduced almost twice that of those foci containing 2-3 aberrant crypts. When interpreted in the context of the results of Pretlow et al, it is likely that this inhibition will translate into an inhibition of tumor formation when the animals are examined at later time points.

Example 3: Influence of Oxidation Products of 5-ASA on in vitro colon cancer cell proliferation.

Although the inhibition seen with balsalazide in the examples above was identical between both cell types, LS174T cells consistently appeared to be more sensitive to the actions of 5-ASA than HT-29 cells. In addition, the inhibitory response of both cell types to 5-ASA varied between individual experiments from a maximum of 70% to a minimum of 10%. In designing experiments to determine the source of the variability, it was determined that 5-ASA stock solutions which were made up a minimum of 3 days prior to addition to the cells consistently produced a greater inhibitory effect. This difference is demonstrated in Figure 7.

In this experiment the growth of LS174T cells exposed to freshly dissolved 10 mM 5-ASA showed a growth response over 4 days which was 82.7% of that displayed by control cells. In contrast, the growth of parallel cultures exposed to 10 mM 5-ASA which had been dissolved in culture media 4 days prior to the start of the experiment showed a growth response over 4 days which was only 22.9% of that displayed by control cultures. This striking difference suggested that the 5-ASA was being converted to a more active metabolite while in the presence of certain media components.

One of the important activities associated with 5-ASA is its anti-oxidant properties. Indeed certain of its

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therapeutic benefits in the treatment of intestinal inflammation are thought to be derived from its ability to scavenge free radicals including O_2^- released from inflammatory cells and thereby reduced oxidative damage.

- 5 This suggested that the changes observed in the inhibitory activity associated with 5-ASA when pre-incubated in tissue culture media could be consistent with an oxidation event. To test this possibility, 5-ASA was subjected to oxidation using sodium hypochlorite at various ratios and the
- 10 subsequent cell growth inhibitory activity then assessed. As shown in Table III below, increasing amounts of hypochlorite resulted in greater 5-ASA inhibitory activity.

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TABLE III
Increase in 5-ASA Growth Inhibitory Activity Following
Hypochlorite Exposure

5-ASA (mM)	Na Hypochlori te (mM)	*Increase in Growth Inhibition (%)
10	0	0
10	0.01	2
10	0.05	4
10	0.1	8
10	0.2	26
10	0.3	42
10	0.4	48

* Basal inhibitory activity by unoxidized 5-ASA was 45%.
Background inhibitory activity by 0.4mM NaOCl was less
than 2%.

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Previous studies by others have shown that oxidizing conditions such as exposure to hypochlorite or hydrogen peroxide can give rise to several different 5-ASA metabolites. Two such oxidation products, gentisic acid (Dull et al. (1987) Biochem. Pharmacol. 36:2467-2472) and 5-nitro-salicylate (Laffafian et al. (1991) Biochem. Pharmacol. 42:1869-1874) have been elucidated. In view of the results above, both of these oxidation products of 5-ASA were tested for colon cancer cell growth inhibitory activity. As shown in Table IV below using four different human colon cancer cell lines, both metabolites inhibited cell growth with the 5-nitro derivative appearing to be more potent. The relative potencies of balsalazide, its primary metabolite, 5-ASA and two of the possible 5-ASA oxidation products on growth inhibition of each of the cell lines are shown in summary form in Table IV below.

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Table IV

IC₅₀ of Balsalazide and metabolites for inhibition of human colon cancer cell proliferation. Concentrations are in mM

C e l l Line	Balsalaz ide	5-ASA	5-ASAox	Gentisa te	5-NSA
LS174T	5.29	11.56	6.55	5.85	2.69
HT-29	5.71	11.89	5.91	5.05	1.63
LoVo	5.00	10.21	5.44	4.42	1.38
HRT-18	3.88	10.49	6.98	2.98	1.46

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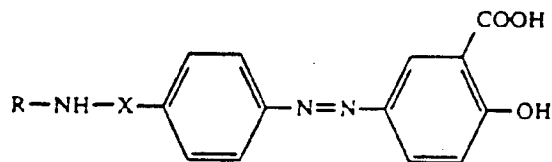
All publications and patent applications cited in this specification are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated
5 by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

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WHAT IS CLAIMED IS:

1. A method of treating an individual suffering from colon cancer or at risk to develop colon cancer comprising administering to the human an effective amount of a pharmaceutical composition comprising a 2-hydroxy-5-phenylazobenzoic acid derivative of the general formula:



wherein X is an -SO₂- or -CO- group and R is either a phenyl or carboxymethylphenyl radical or is a radical of the formula - (CH₂)_n-Y, in which Y is a hydroxyl group, an amino group, a monoalkyl- or dialkyl-amino group, the alkyl moieties of which contain up to 6 carbon atoms, or a carboxylic or sulphonic acid group, and n is a whole number of from 1 to 6, and in which one or more of the hydrogen atoms in the alkylene radical can be replaced by amino groups, monoalkyl- or dialkyl-amino groups, the alkyl moieties of which contain up to 6 carbon atoms, or alkyl radicals, and in which the -(CH₂)_n-Y radical is either attached directly to the nitrogen atom or via a benzene ring, with the proviso that R-NH-X- is other than a -CO-NH-CH₂-COOH radical; or an ester or active metabolite or an oxidation product of an active metabolite thereof, or a non-toxic, pharmacologically acceptable salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof.

2. A method according to claim 1 wherein the pharmaceutical composition consists essentially of the 2-

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hydroxy-5-phenylazobenzoic acid derivative or ester or active metabolite or an oxidation product of an active metabolite thereof or a salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof in admixture with a solid or liquid pharmaceutical diluent or carrier.

3. A method according to claim 1 wherein the 2-hydroxy-5-phenylazobenzoic acid derivative is balsalazide.

4. A method according to claim 1 wherein the active metabolite is 5-ASA.

5. A method according to claim 1 wherein the oxidation product of an active metabolite is an oxidation product of 5-ASA.

6. A method according to claim 5 wherein the oxidation product of 5-ASA is gentisic acid.

7. A method according to claim 5 wherein the oxidation product of 5-ASA is 5-nitro-salicylate.

8. A method of claim 1 wherein the pharmaceutical composition is administered orally to an individual suffering from or at risk to develop colon cancer in a daily dosage ranging from 1 to 14g per 70 kg body weight per day of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof or a salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof.

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9. A method of claim 1 wherein the pharmaceutical composition is administered rectally to an individual suffering from or at risk to develop colon cancer in a daily dosage ranging from 1 to 14g per 70 kg body weight per day of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof or a salt of the 2-hydroxy-5-phenylazobenzoic acid derivative or an ester or an active metabolite or an oxidation product of an active metabolite thereof.

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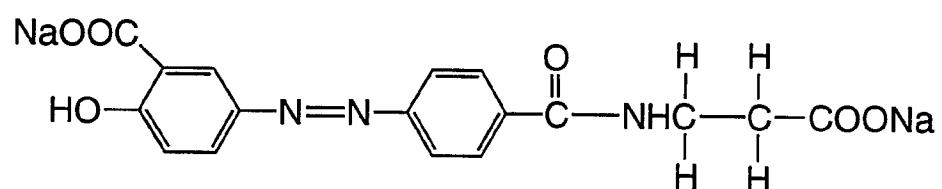


FIG. 1

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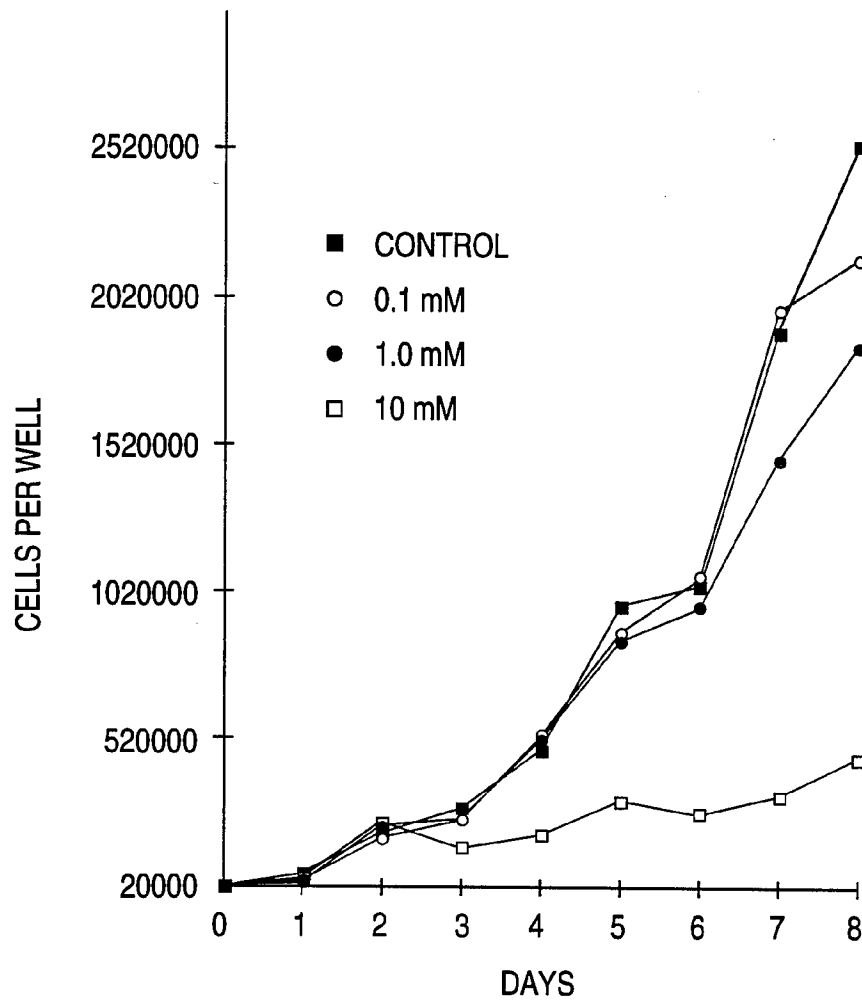


FIG. 2

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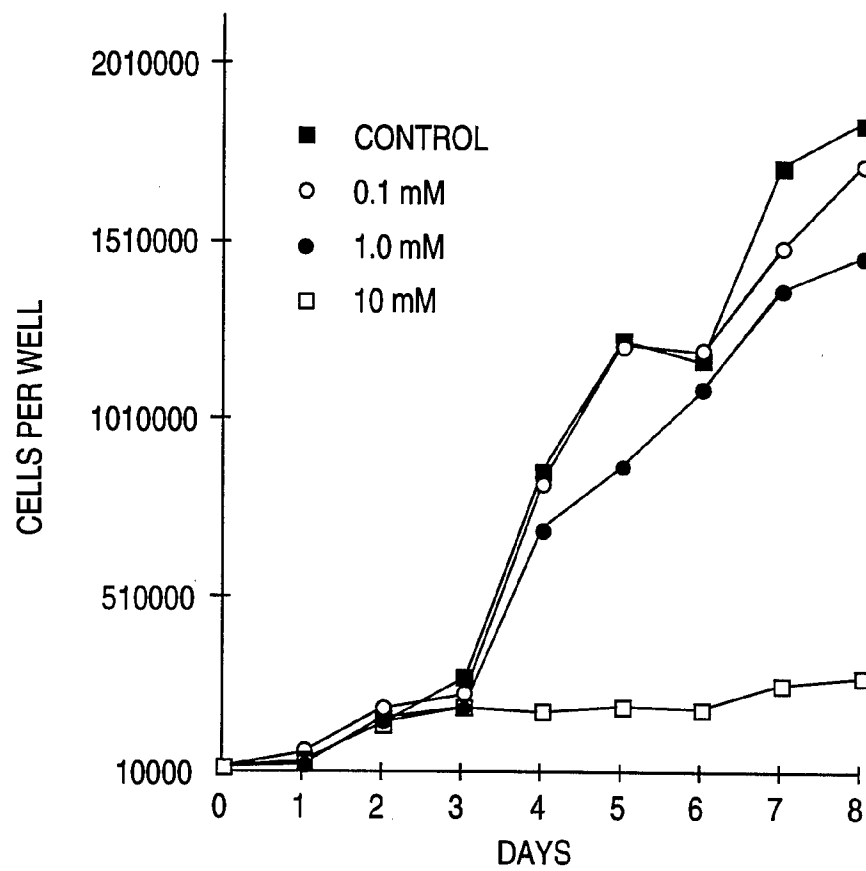


FIG. 3

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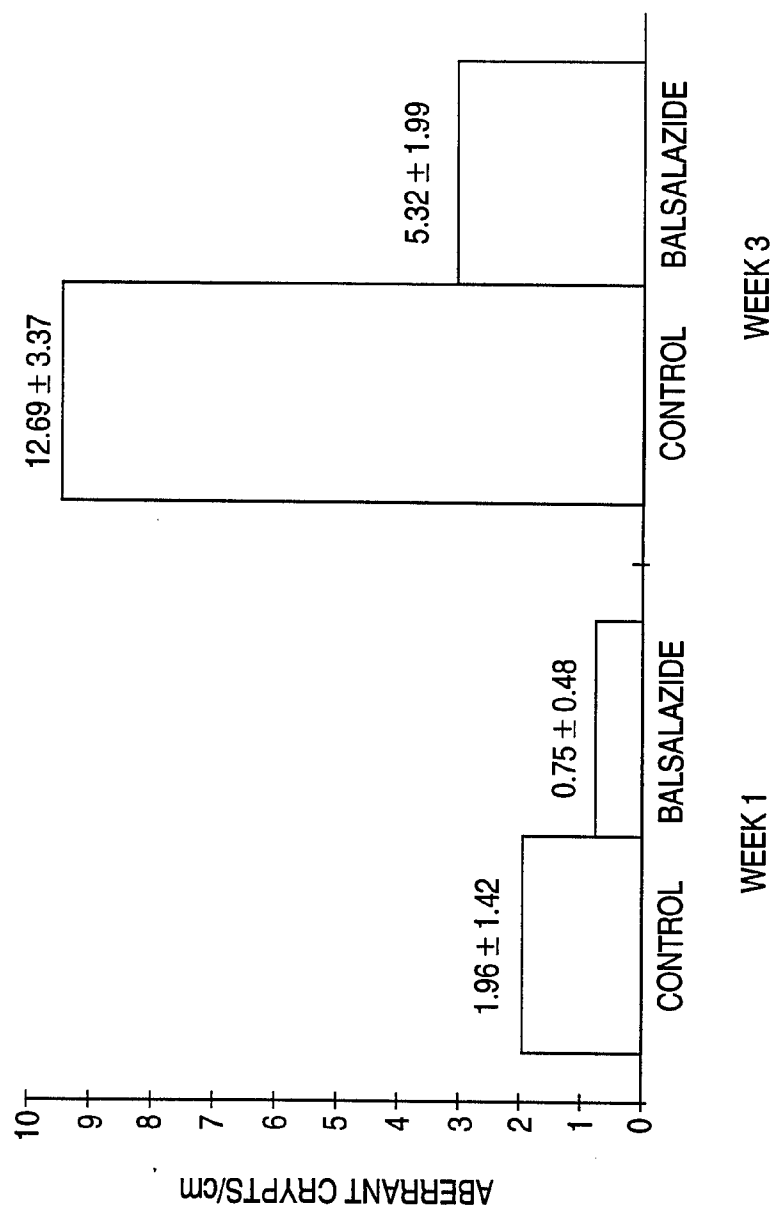


FIG. 4

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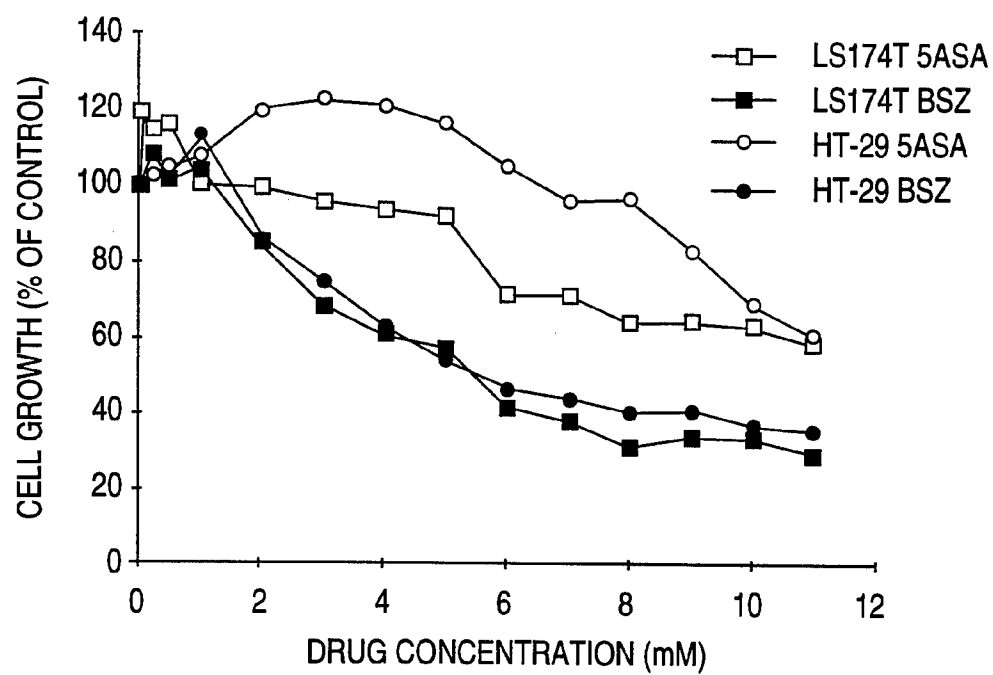


FIG. 5

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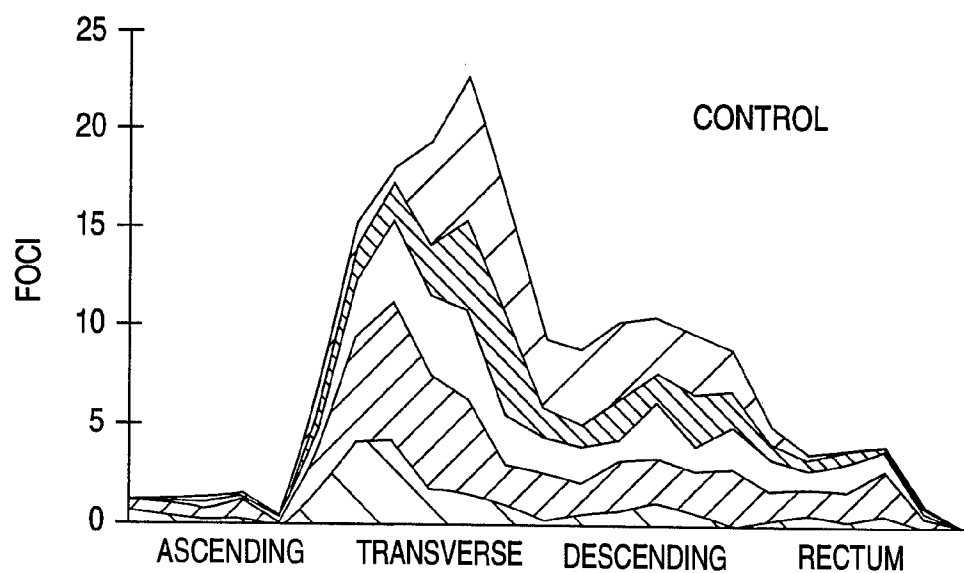


FIG. 6A

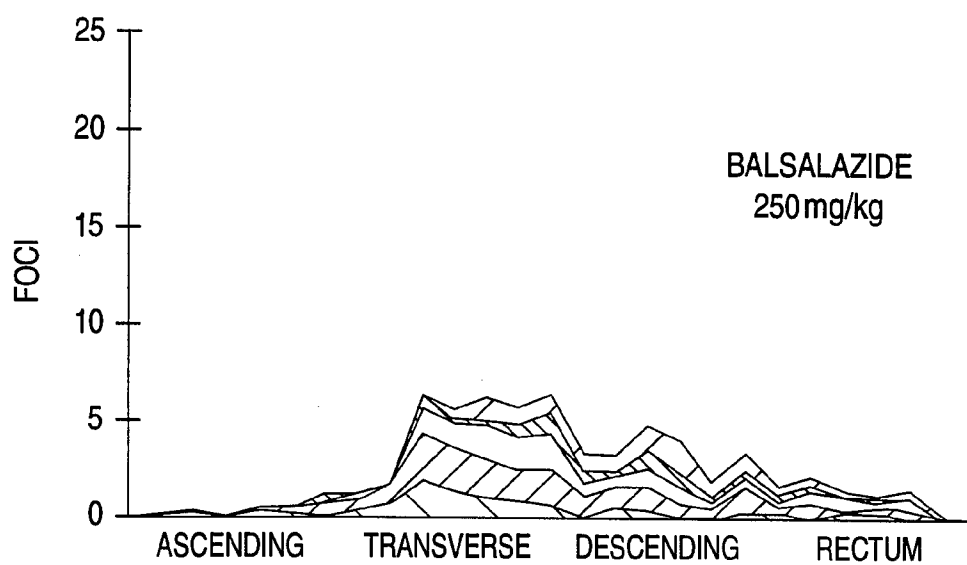
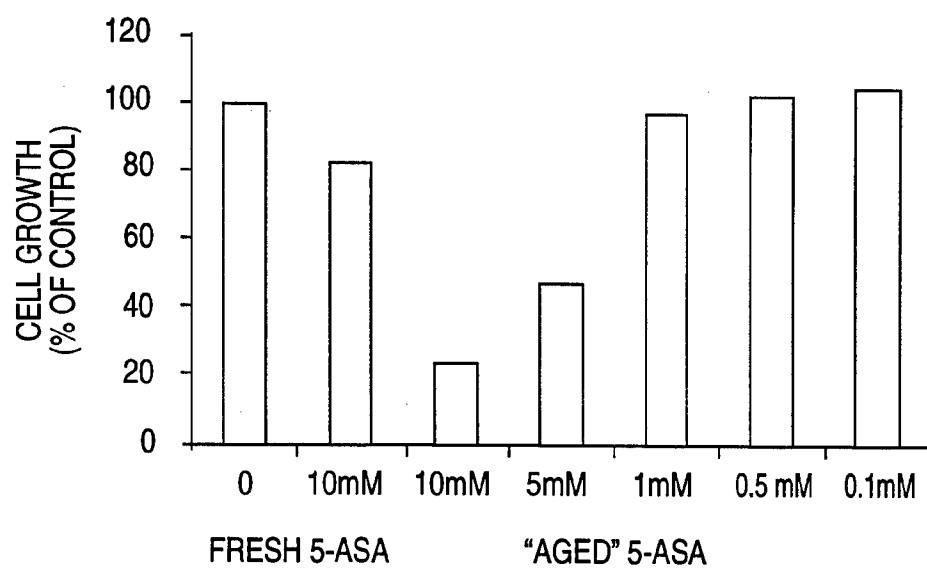


FIG. 6B

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**FIG. 7**

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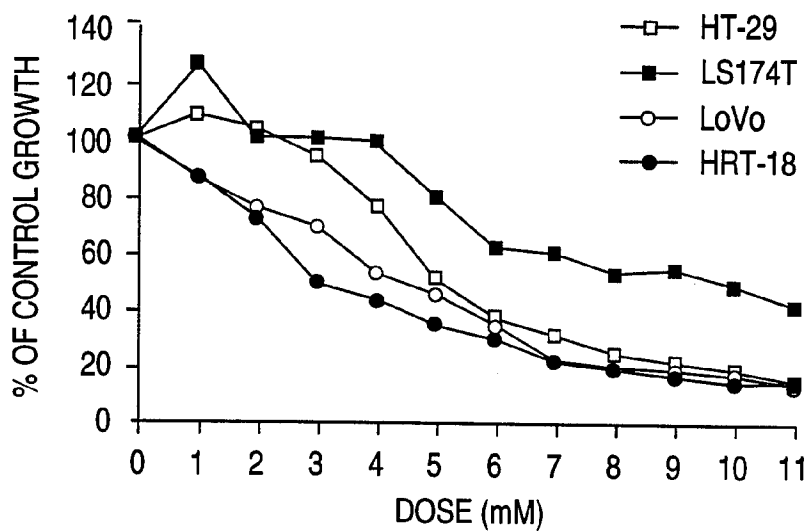


FIG. 8A

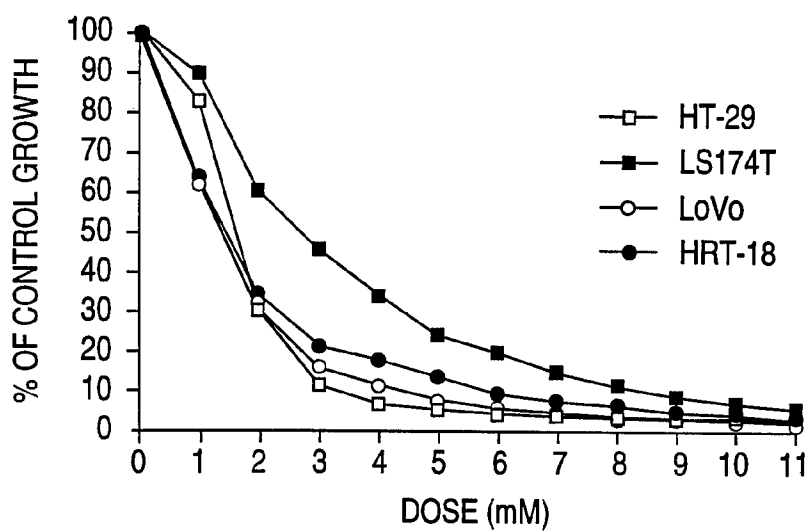


FIG. 8B

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US95/00055

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/655, 31/615

US CL :514/150, 166

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/150, 166

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,412,992 (CHAN) 10 NOVEMBER 1983, see entire document.	1-9
Y	"STEDMAN'S MEDICAL DICTIONARY", 24th Ed., published 1983 by Williams & Wilkins (Baltimore, MD), page 297, see "colitis, ulcerative".	1-9

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

11 APRIL 1995

Date of mailing of the international search report

03 MAY 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

JEROME D. GOLDBERG jd

Telephone No. (703) 308-1235